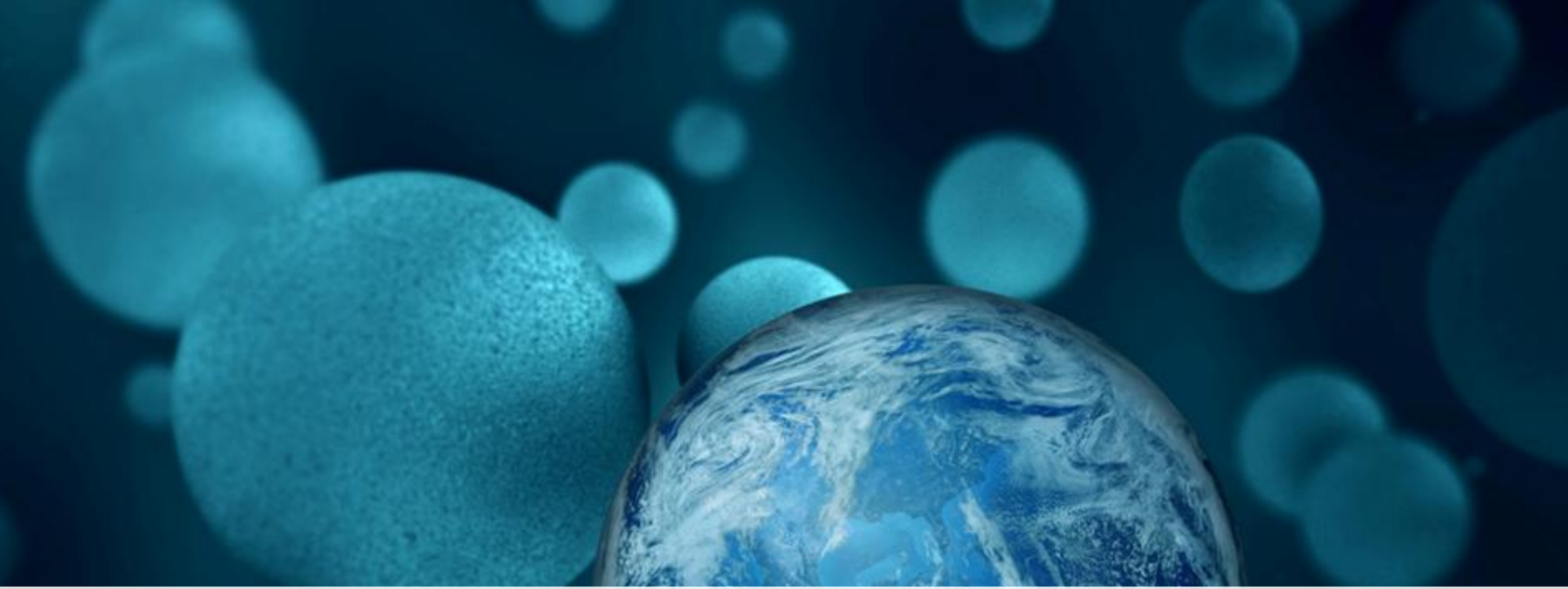


Automated Sample Preparation Tools in Routine Laboratory Practice

Elsamoul Hamdnalla
Thermo Fisher Scientific

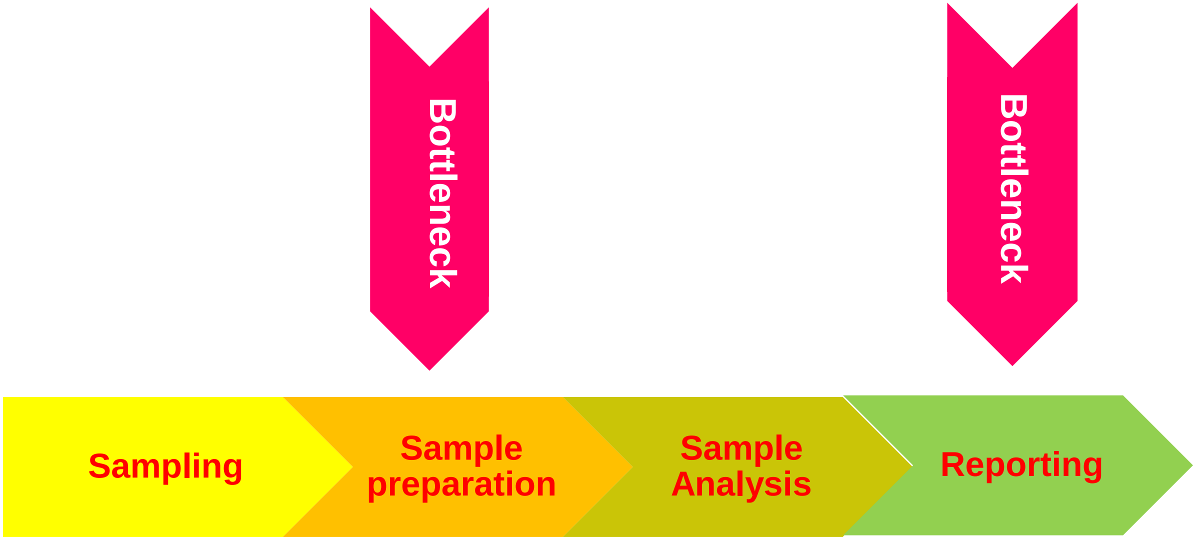
The world leader in serving science

Outline

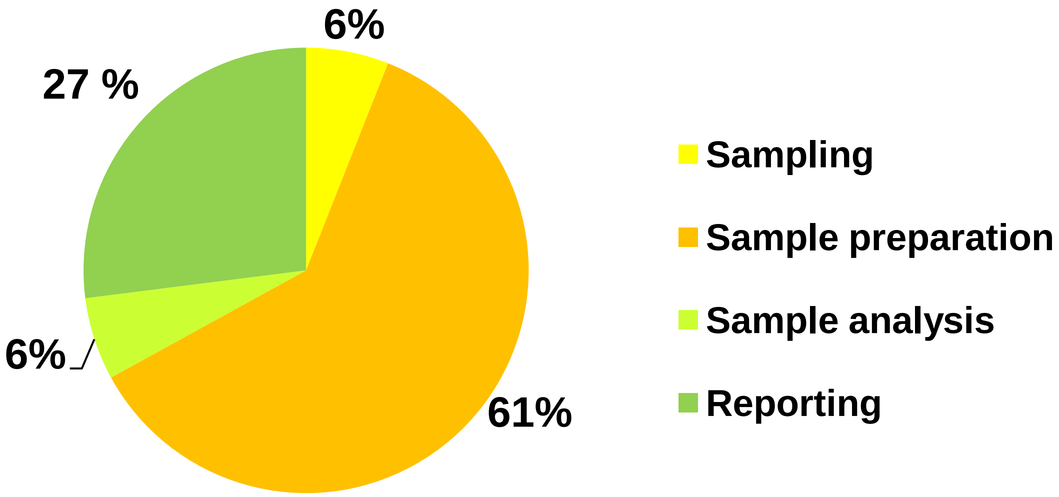
- Overview Sample Preparation
- Automated Sample Preparation in Ion Chromatography
- Automated Sample Preparation in GC/LC
- Accelerated Solvent Extraction
- Introduction to Liquid – Solid Extraction
- Introduction to the ASE 350
- ASE Applications Overview

Workflow for analysis of pesticide residues

Very rarely can analytical samples be analyzed directly without prior treatment



Sample preparation is a labor-intensive and time-consuming work



Automated sample preparation in IC … ☺

In the Press…

21 February 2011 Volume 7 Issue 3

The Column

the interactive e-publication for the global separation science industry

How good is your sample preparation?

AN ADVANSTAR PUBLICATION

When do you need sample pretreatment?

- No better column for the job
- No alternative detector
- Shortened retention times, poor peak efficiency
- Poor resolution
- Poor reproducibility
- Electrochemical detector electrode fouling
- Irregular baseline
- Interference with analyte signal
- High-Low ratio is too high

Manual sample preparation – cartridges

Interfering Ions

Poor IC performance
Short consumables lifetime
High cost of ownership

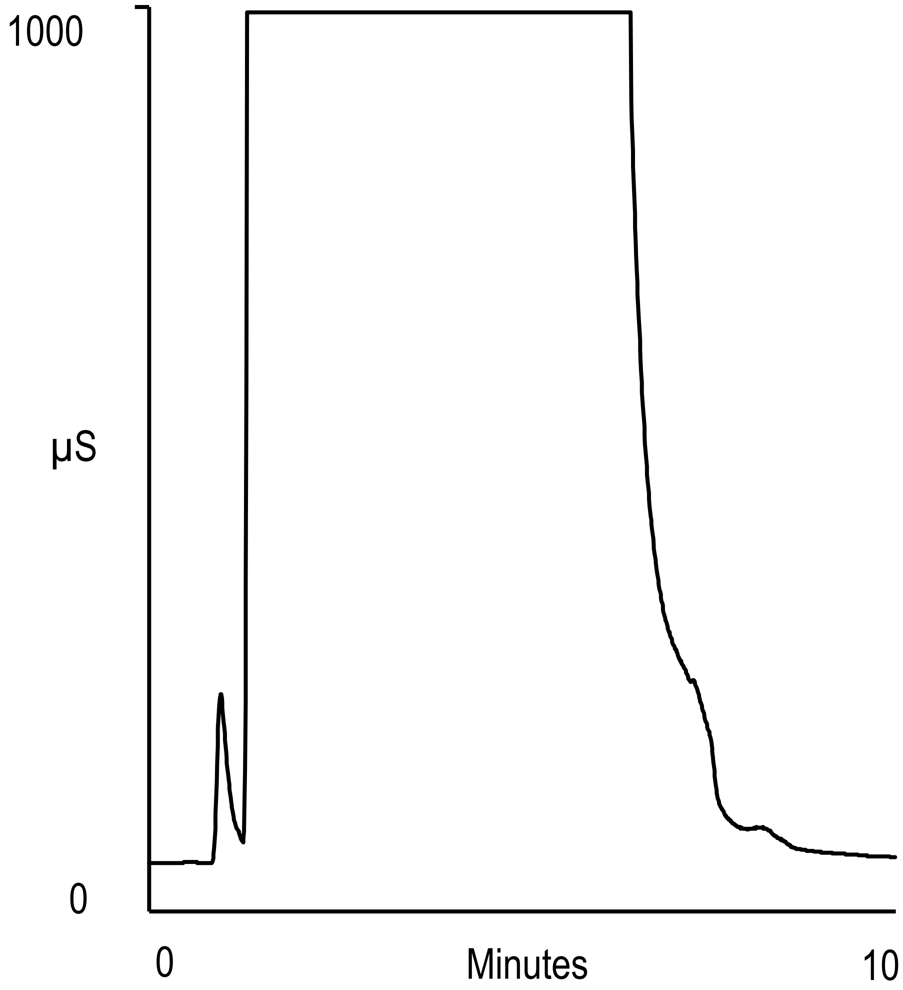
Solution—Dionex Matrix Elimination Cartridges

Eliminate interfering ions
Automated and manual methods
Superior IC performance
Extended lifetime of consumables
Lower cost of ownership

Sample pretreatment phase chemistries

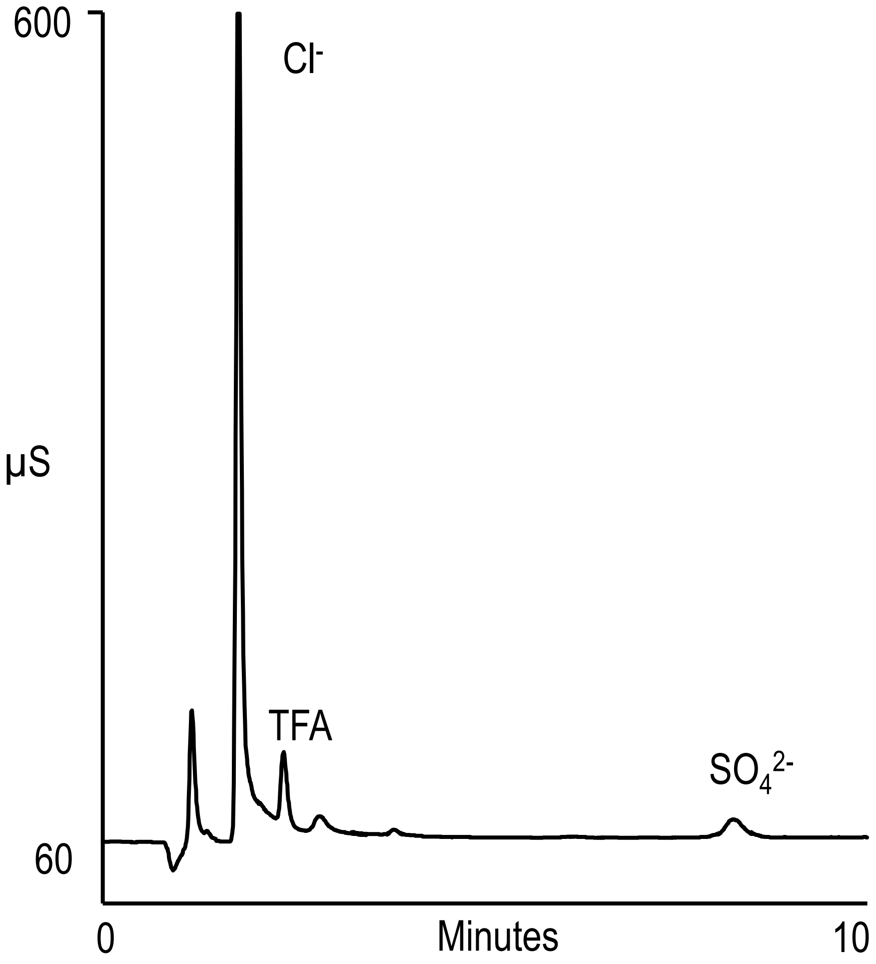
Phase	Functionality	Retention Mechanism	Application
A	Anion-exchange Bicarbonate form	Ion exchange	Remove anions, increase pH
Ag	Sulfonated resin Silver form	Ion exchange and Precipitation	Remove chloride
Ba	Sulfonated resin Barium form	Ion exchange and Precipitation	Remove sulfate
H	Sulfonated resin Acid form	Ion exchange	Remove cations, reduce pH
M	Iminodiacetate Ammonium form	Chelation	Remove transition metals
Na	Sulfonated resin Sodium form	Ion exchange	Remove cations, no pH change
P	Poly-vinylpyrrolidone	H-bonding/ Complexation	Remove phenols, azo dyes, humic acids
RP	Poly-divinylbenzene	Adsorption	Remove neutral hydrophobics

Determination of TFA in 20% HCl



Column:	IonPac AS4A-SC with Guard
Eluant:	1.7 mmol/L $NaHCO_3$ 1.8 mmol/LNa_2CO_3
Flow:	2 mL/min
Detection:	Suppressed Conductivity
Injection vol.:	25 µL

Determination of TFA in 20% HCl

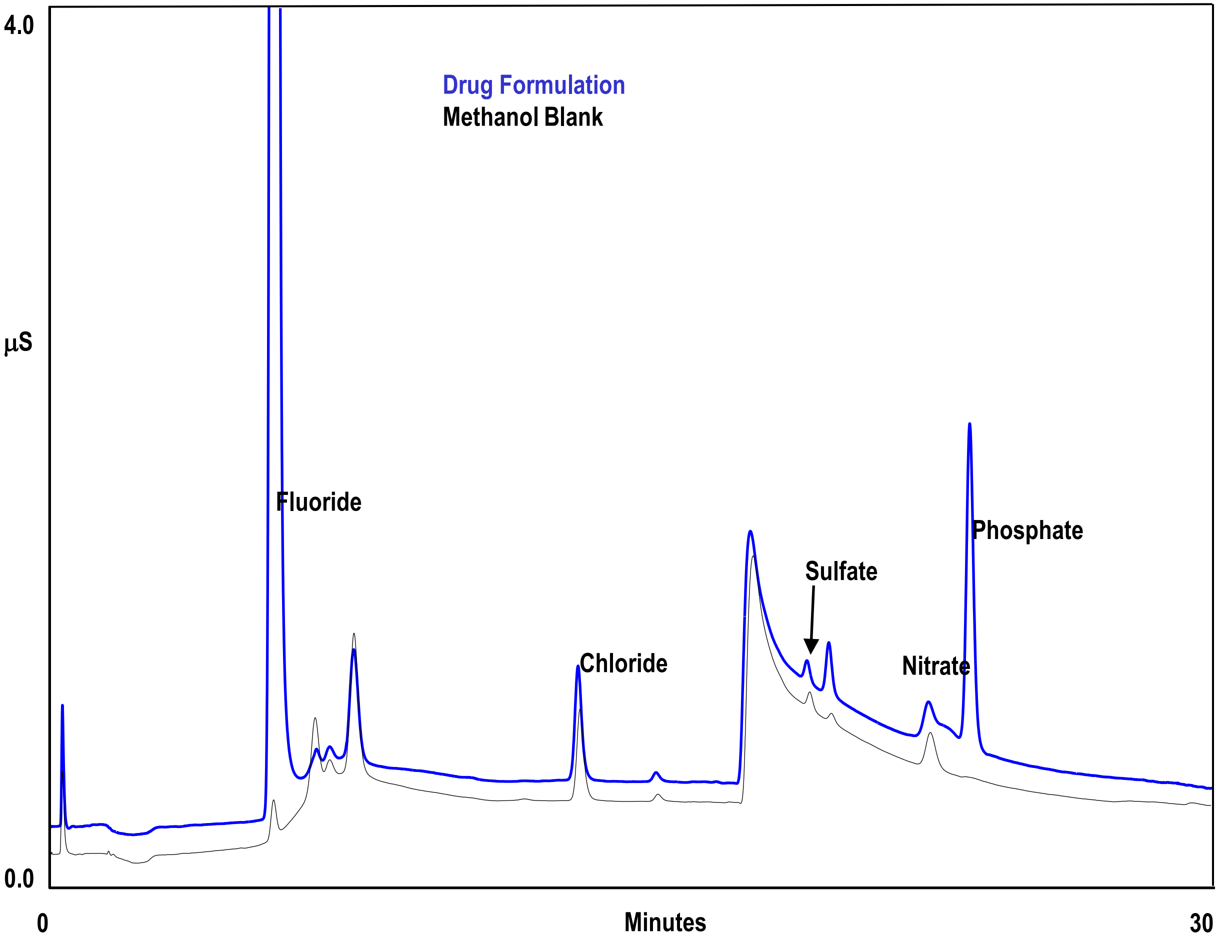


Column:	IonPac AS4A-SC with Guard
Eluant:	1,7 mmol/L $NaHCO_3$ 1,8 mmol/LNa_2CO_3
Flow:	2 mL/min
Detection:	Suppressed Conductivity
Injection vol.:	25 µL
TFA Concentration in original Solution:	3.7 mg/L

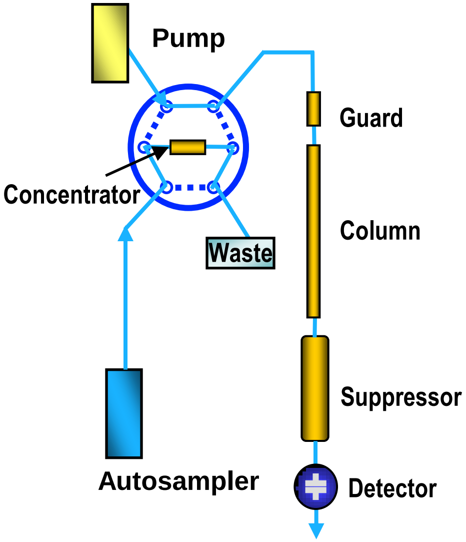
Automated sample preparation

- Anions in Organic Solvents
- Anions and Cations in Hydrogen Peroxide
- Anions in Weak Acids
- Anions and Cations in Acids and Bases
- Removing Dissolved Organic Matter
- Automation Using Dionex InGuardCartridges
- Inline Filtration
- pH and Conductivity Measurement
- Summary

Anions from water insoluble drug



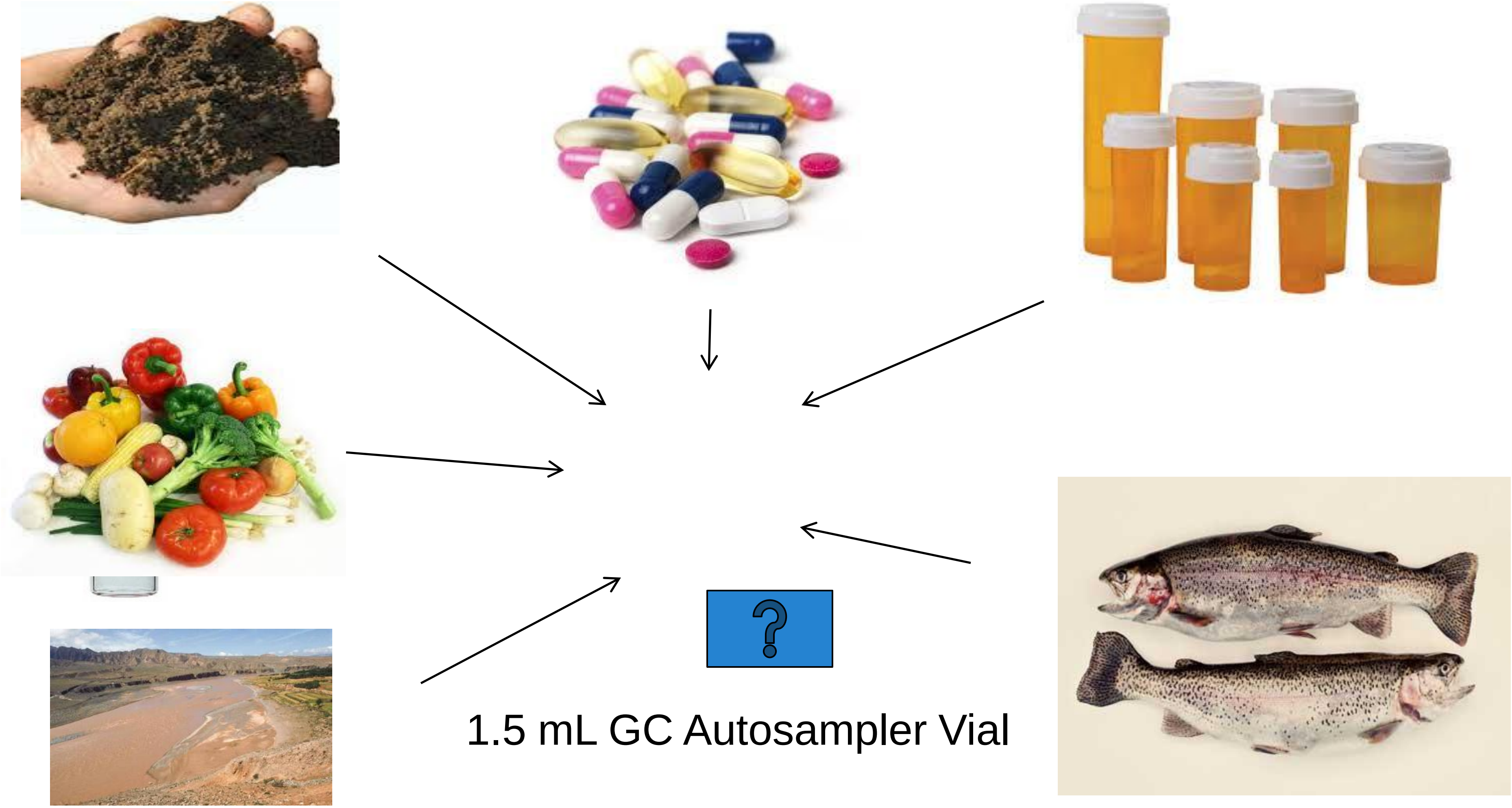
Instrument:	Thermo Scientific ™Dionex™ ICS-3000 and AS
Concentrator:	UTAC-ULP1 (5 x 23 mm)
Column:	Thermo Scientific ™Dionex ™ IonPac™ AG15 (mm)/ AS15 (2 mm)
RFIC-EG:	KOH Gradient
Temperature:	30° C
Flow Rate:	0.4 mL/min
Inj. Vol.:	100 µL
Detection:	Suppressed conductivity
Sample:	Drug formulation in Methanol
Procedure:	1. Sample passed through UTAC 2. Anions collected; drug passed to waste 3. UTAC flushed with water from AS to remove MeOH 4. Anions injected onto column



Anions Trapped and Drug Substance Removed

Automated sample preparation in GC/LC

The Challenge for Analysis



1.5 mL GC Autosampler Vial

How do we get analytes out of these samples?

The answer is sample preparation

- Extraction
  - Removes analytes from the sample
  - Eliminates compounds that interfere with the analysis (Clean Up)

- Evaporation
  - Concentrates extracted analytes for analysis
  - Evaporates extracted samples for re-constitution

- Most time consuming part of analytical procedure (>60%)*

- Single largest source of errors in the workflow (>30%)**

*Majors, R.E. *LC-GC*, **1995**, *13*, 742-749, and **Majors, R.E. *LC-GC*, **1999**, *17*, S8 - S13

Extraction techniques

- Liquid Liquid extraction
- Solid liquid extraction (Quechers)
- Pressurized extraction (ASE)
- Heated extraction – soxhlett

- **Scope:**
  - Extracting of target analytes from matrix with high recovery
  - Removal of matrix

Now . . . Accelerated Solvent Extraction - ASE™

- Automates sample preparation for solid and semisolid samples using solvents at elevated temperatures
- Operates above the boiling point of extraction solvents by using elevated pressure
- Walk-away system that extracts and clean up to 24 samples unattended
- Well established and proven technique that is superior to Soxhlet and approved for U.S. EPA Method 3545A

Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system

How does Accelerated Solvent Extraction work?

Cell loaded into oven

↓ 1 min

Fill, heat, equilibrate

↓ 5-9 min

Static extraction

↓ 3-5 min ↕ static cycle

Fresh solvent rinse

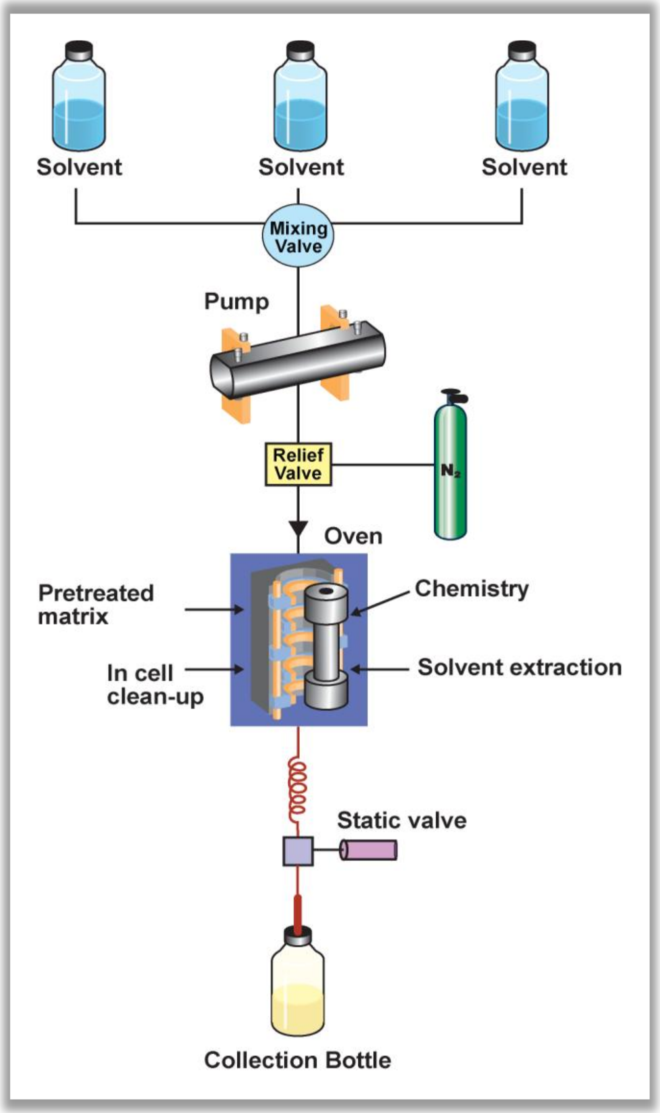
↓ 0.5 min

Nitrogen purge

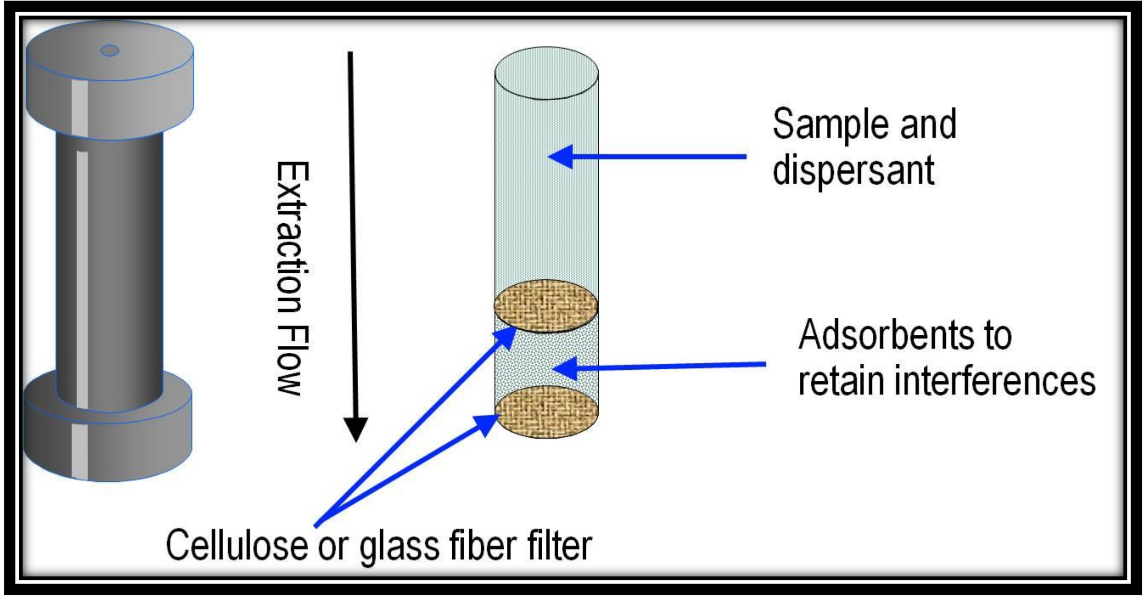
↓ 1-2 min

Filtered extract

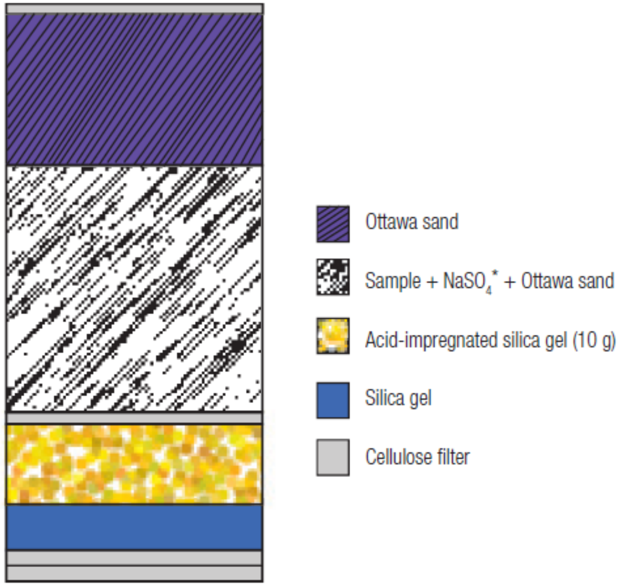
15-20 min Total Time



ASE in-line clean up + use of adsorbents improves selectivity

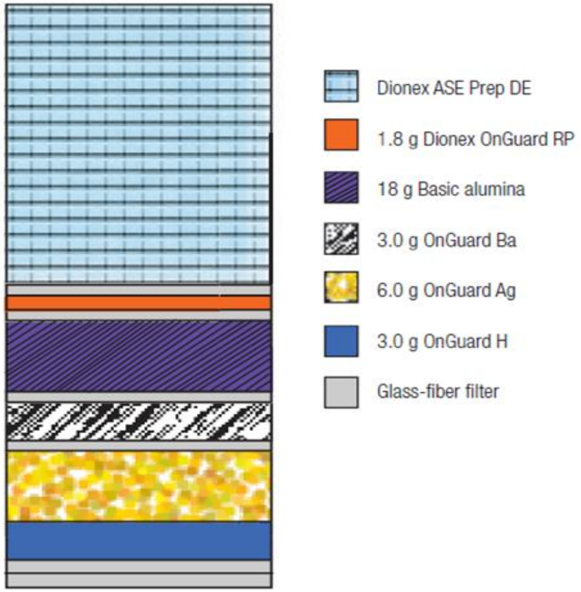


Schematic of the Cell



Preparation of the Extraction Cell for the Selective Extraction of PCBs from Fish Meal

Preparation of the Extraction Cell for the Selective Extraction of Perchlorate from Vegetation



ASE in-line cleanup

Adsorbent and Uses	
Carbon	Removes organics and nonpolar compounds
Copper	Removes sulfur
Ion-exchange Resins	Removes organics, ionic interferences for IC and IC/MS analysis
C_{18} Resin	Removes organics, polar compounds, lipids, colors
Acid-impregnated Silica Gel	Removes lipids
Alumina	Removes nonpolar lipids, colors
Florisil	Removes nonpolar lipids
Silica Gel	Removes nonpolar lipids

International agency acceptance of ASE

United States

U.S. EPA Method 3545A (OCP, OPP, BNA, TPH, PCDD, herbicides and semi-volatiles)

U.S. EPA Method 8267 (Toxaphene)

U.S. EPA Method 6860 (Perchlorate)

NOAA Method NWFS-NWFSC-59 (Hydrocarbons)

ASTM D-7210 (Polymer Additives)

China

Method GB/T 19649-2006 for 475 pesticides in grains and grain products

Method GB/T 23376-2009, pesticides in tea leaves

Method GB/T22996-2008, ginsenosides in ginseng

Mexico

National Standard NMX-AA-146-SCFI-2008 for PAHs in soils and sediments

Germany

Method L00.00-34 for pesticides in foodstuffs

Global industries using ASE

Water Treatment Plants

Rubber & Polymers

Natural Products

Contract Laboratories

Government Agencies

Dietary Supplements

Biotech/Pharmaceutical

Food and Beverage

Key ASE applications summary

Industry	Analyte	Determinative Step	Matrix	Application Note
Environmental	Polyaromatic Hydrocarbons (PAHs)	GC-MS	Soil, Tissue	AN 1025
	Polychlorinated Biphenyls (PCBs)	GC-ECD	Soil, Tissue, PUFs	AN 1025
	Dioxins and Furans	GC-MS/MS	Sediment, brick, dust, ash	AN 10336
	Total Petroleum Hydrocarbons (TPH)	GC-FID	Soil	AN 324
	Base, Neutral, Acids (BNAs)	GC-MS	Soil	AN 317
Food	Fat Content	Gravimetric	Chocolate Meat Snack Foods Infant Formula	AU 344 AN 334 AN 321 AN 329 AU 195
	Oil Content	Gravimetric	Oil seeds (e.g. canola)	AU 325
	Pesticide Residues	GC-MS	Fruits, Vegetables, Animal Feeds	AN 332 AN 349
	Acrylamide	LC-MS	Coffee, Chocolate	AN 358
Natural Products	Herbal Marker Compounds	LC-UV	Plants	AN 362
	Active Ingredients in Herbal Supplements	LC-UV	Pills	AN 335
Chemical	Polymer Additives	LC-UV	Polymer Materials	AN 331
	Bioalcohol	Gravimetric	Biomass	AN 363
Pharma	Leachables & Extractables	LC-MS/MS	Drug Packaging	AN 71302
	Active Ingredients	LC-UV	Transdermal Patches	AN 327

Summary of U.S. EPA equivalency study for ASE

Compound Class	Comparison Technique	Relative Recovery
Organochlorine pesticides (OCP)	Automated Soxhlet	97.3%
Organophosphorus pesticides (OPP)	Automated Soxhlet	99.2%
Semivolatiles (BNA)	Soxhlet	98.6%
Chlorinated herbicides	Shake method	112.9%
Polychlorinated biphenyls (PCB)	Various reference materials	98.2%
Polycyclic aromatic hydrocarbons (PAH)	Various reference materials	104.8%

Comparison to other techniques (U.S. EPA Methods)

U.S. EPA Method	Technique	Solvent Used Per Sample	Extraction Time Per Sample
3545A	ASE	15 – 45 mL	10-15 min
3540	Soxhlet	300 – 500 mL	18 hours
3541	Automated Soxhlet	50 mL	2 hours
8151	Shaker	300 mL	2 hours
3546	Microwave*	25 mL	15 min

*Requires additional cooling and filtering steps (~ 45 min/sample)

Thermo Scientific AutoTrace 280 SPE instrument

47 mm Disk System

Cartridge (1, 3, or 6 mL) System

The Thermo Scientific™ AutoTrace 280 provides reliable automated SPE for analytical chemists determining organic pollutants in large-volume aqueous samples. Unlike traditional methods such as liquid-liquid extraction using separatory funnels, the AutoTrace 280 saves time, solvent and labor ensuring high reproducibility and productivity for analytical laboratories. The unit can process up to 6 samples in 2-3 hours.

AutoTrace 280: for liquid samples

- **Reduced sample extraction cost**
  - Solvent consumption (up to 90% less than LLE)
  - Labor cost (15 min operator intervention)
- **Improved productivity**
  - 6 samples loaded onto cartridges in 15 min
- **Improved analytical precision**
  - Automated sample loading and elution
  - Positive pressure displacement

6 mL Cartridge System

Pesticide recovery study

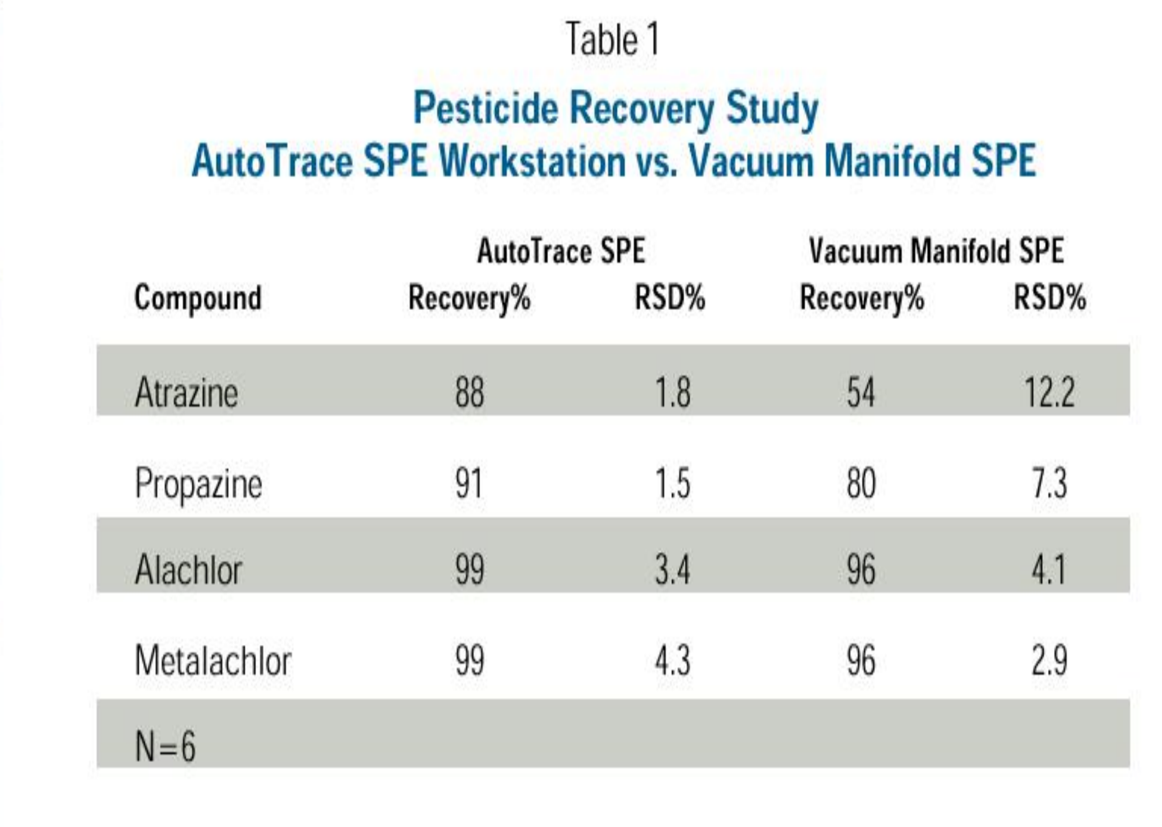


Table 1

Pesticide Recovery Study
AutoTrace SPE Workstation vs. Vacuum Manifold SPE

	AutoTrace SPE		Vacuum Manifold SPE	
Compound	**Recovery%**	**RSD%**	**Recovery%**	**RSD%**
Atrazine	88	1.8	54	12.2
Propazine	91	1.5	80	7.3
Alachlor	99	3.4	96	4.1
Metalachlor	99	4.3	96	2.9
N=6				

AutoTrace 280 Produces Higher Recoveries than Vacuum Manifold

Key AutoTrace 280 applications summary

Analytes	Determinative Step	Matrix	Application Note
Polyaromatic Hydrocarbons (PAHs)	GC-MS	Surface Water	AN 876
Dioxins and Furans	GC-MS	Surface Water	AB 805
Polychlorinated Biphenyls (PCBs)	GC-ECD	Surface Water	AB 805
Endocrine Disruptors	GC-MS	Surface Water Drinking Water	AB 801
Semivolatile Organic Compounds	GC & GC-MS	Drinking Water	AN 819
Organochlorine Pesticides	GC-ECD	Drinking Water	AN 1004
Sex Hormones	HPLC-UV	Drinking Water	TN 148
Tricolsan	HPLC-UV	Drinking Water	AN 1081
Explosives	HPLC-UV	Ground Water	AN 358
Linear Alkyl Benzene Sulfonate	HPLC-UV	Waste Water	AN 1080
Organophosphorous Pesticides	GC-NPD	Drinking Water	AN 1097 (Pending Publication)

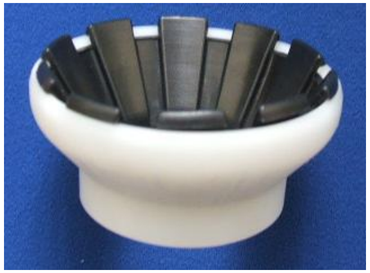
Visit www.thermofisher.com/samplepreparation

The Thermo Scientific Rocket: for sample evaporation

- Centrifugal evaporator the uses low temperature boiling
- Evaporates to dryness and concentrates to a fixed volume
- Allows direct transfer of 60 mL ASE vials to minimize sample handling
- Automatic end point detection
- Preprogrammed methods provide walk-away capability

Flasks for evaporating or concentrating

- 450 mL Evaporation Flask
- Used for evaporating to dryness



- 400 mL flask that concentrates sample into GC autosampler vials
- Vial is insulated so that only solvent in flask evaporates

60 mL ASE vials for direct transfer

- Pucks accept 60 mL ASE vials
- Each puck accepts 3 vials
  - Total capacity per system is 18 vials
- Allows direct sample transfer from the ASE 350 system

- GC Vial is inserted into the flip flop funnel
- GC vial is insulted to prevent boiling of solvent
- ASE vial is inserted directly into the Rotor
- GC vial is placed directly into autosampler once complete

60 mL ASE Vial Flip Flop

What about solvent recovery? & How fast does it go?

Solvent	Recovery
DCM	80%
DMF	99%
Ethanol	99%
Methanol	98%
Water	99%
Water/acetonitrile	98%

	100 mL	250 mL	450 mL
DCM	10 min	20 min	35 min
Methanol	20 min	45 min	1.5 h
DMF	30 min	1 h	2 h
Water	35 min	1.5 h	2 h
Water/ACN (1:1)	1 h	1.5 h	3 h

Times are given for complete dryness for 6 flasks simultaneously evaporated at 40 ºC

Where can I find out more?

GC Farms Customer Testimonial: Accelerated Solvent Extraction and Ion Chromatography

Customer Testimonials

C&EN
CHEMICAL & ENGINEERING NEWS

Serving The Chemical, Life Sciences & Laboratory Worlds

Search

C&EN WEBINARS

Stronger bonds.

Improving Dioxin Extraction Using In-Cell Pressurized Liquid Extraction: A Case Study for Solid Sample Matrices

Thursday December 12th 2013
8:00a.m. PST / 11:00a.m. EST / 16:00 GMT

SPEAKERS

Sascha Usenko,
Assistant Professor of Environmental Product Manager — Dionex ASE Science,
Baylor University

Aaron Kettle,
Product Manager,
Dionex ASE Systems,
Thermo Fisher Scientific

MODERATOR

Linda Wang,
Senior Editor, C&EN

OVERVIEW

In recent years, there has been an increasing demand for high-throughput analytical methods capable of quantifying organic contaminants in biological or other solid matrices. One approach to increasing the throughput is to reduce or eliminate the bottlenecks associated with sample preparation.

WHO SHOULD ATTEND

- Academic or governmental research groups that routine measuring organic contaminants in biological or solid matrices

C&EN
Find us on Facebook
facebook.com/cenews

CHEMISTRY JOB LISTINGS

Chemist | Sonneborn

Scientist - Bioprocess Purification Research and Development | Avitide, Inc.

Tenure-track Assistant Professor in Chemistry & Chemical Biology, Harvard University | Harvard University

Tenure-Track Faculty Positions - Chemistry | Texas A&M University

More jobs >>

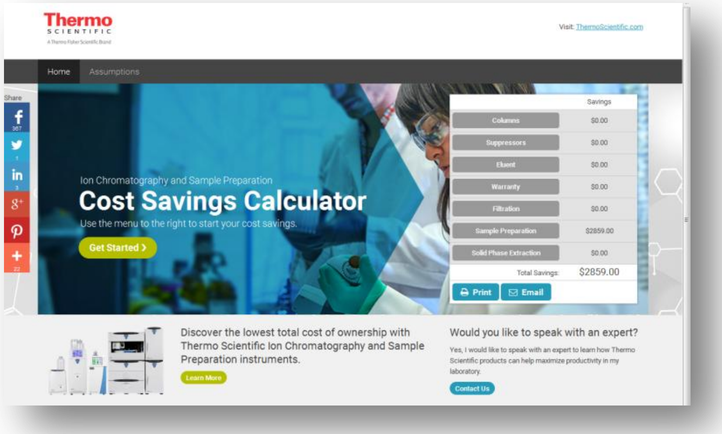
ACS Careers >>

Advertisement

Info for Advertisers

Educational Webinars

Three Year Warranty



Visit: ThermoScientific.com

Ion Chromatography and Sample Preparation

Cost Savings Calculator

Use the menu to the right to start your cost savings.

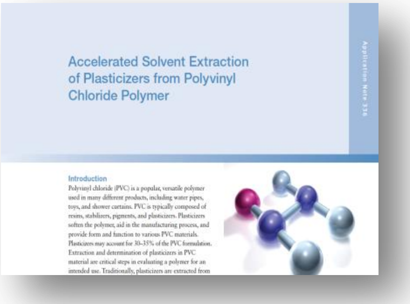
	Savings
Columns	$0.00
Suppressors	$0.00
Eluent	$0.00
Warranty	$0.00
Filtration	$0.00
Sample Preparation	$2859.00
Solid Phase Extraction	$0.00
Total Savings:	$2859.00

Discover the lowest total cost of ownership with Thermo Scientific Ion Chromatography and Sample Preparation instruments.

Would you like to speak with an expert?

Yes, I would like to speak with an expert to learn how Thermo Scientific products can help maximize productivity in my laboratory.

Cost Savings Calculator

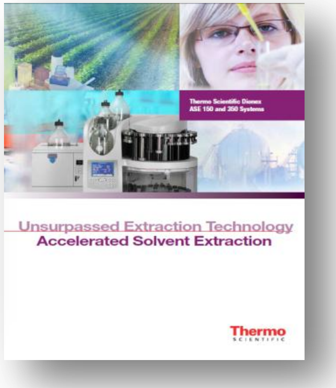


Accelerated Solvent Extraction of Plasticizers from Polyvinyl Chloride Polymer

Application Note 336

Introduction

Polyvinyl chloride (PVC) is a popular, versatile polymer used in many different products, including water pipes, toys, and shower curtains. PVC is typically composed of resins, stabilizers, pigments, and plasticizers. Plasticizers soften the polymer, aid in the manufacturing process, and provide form and function to various PVC materials. Plasticizers may account for 30–35% of the PVC formulation. Extraction and determination of plasticizers in PVC material are critical steps in evaluating a polymer for an intended use. Traditionally, plasticizers are extracted from



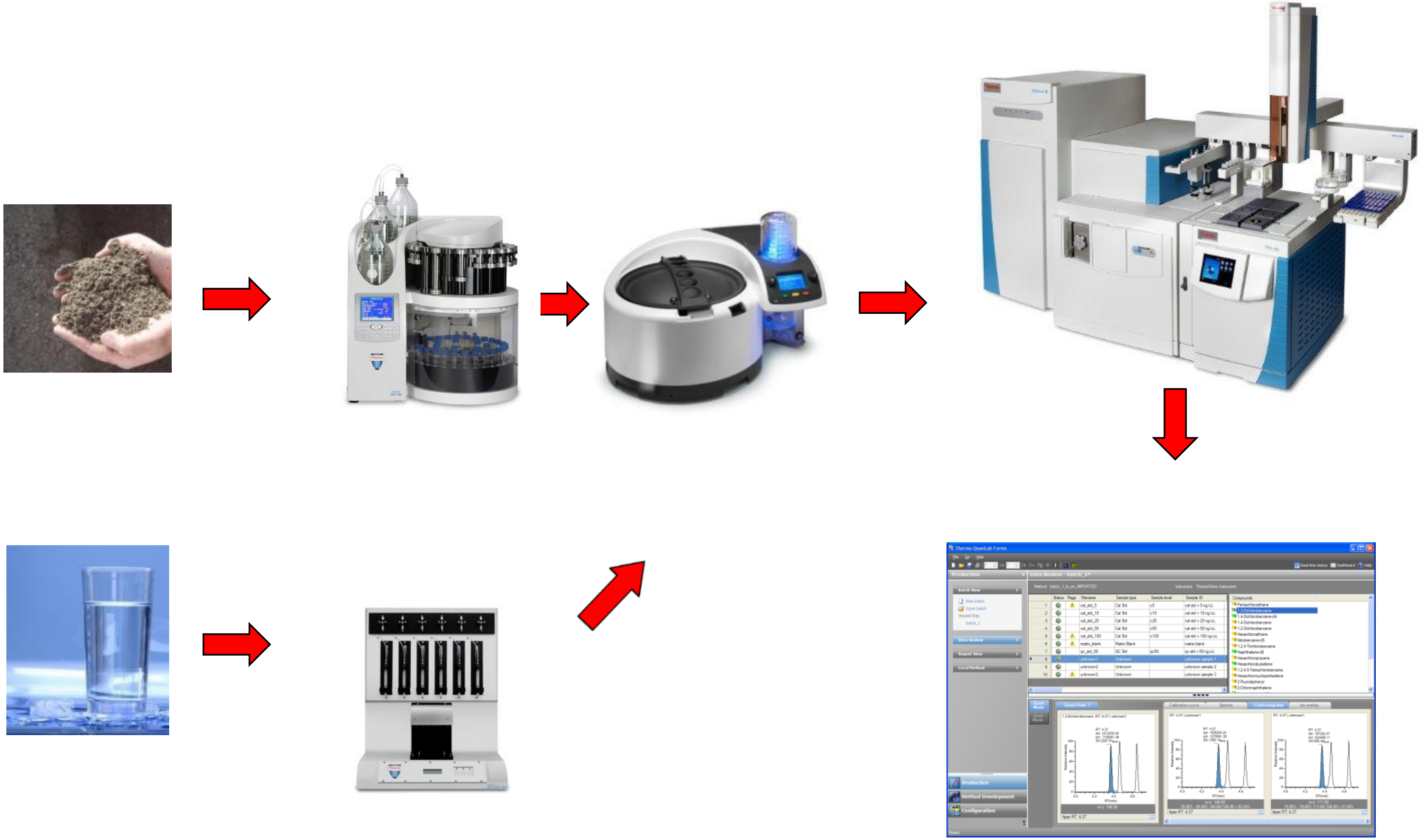
Thermo Scientific Dionex ASE 150 and 350 Systems

Unsurpassed Extraction Technology
Accelerated Solvent Extraction

Application Notes & Brochures

www.thermofisher.com/samplepreparation

Total workflow with fast simplified sample prep



Integrated Workflow Solutions for Sample Preparation

Thermo Fisher
SCIENTIFIC

The world leader in serving science